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Research paper

# *Ovar-DRB1* haplotypes *2001 and *0301 are associated with sheep growth and ewe lifetime prolificacy

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ABSTRACT

*Background:* The major histocompatibility complex (MHC) is an organized cluster of tightly linked vertebrate genes with immunological and non-immunological functions. While the important MHC gene *DRB1* has been examined in regard to many sheep infectious disease traits, only one study, based on microsatellite markers, has previously examined *DRB1* and sheep production traits. Furthermore, to our knowledge no studies have examined *DRB1* relationship with lifetime ewe prolificacy traits. Therefore, we analyzed association between the presence of *DRB1* SNP haplotypes with internationally recognized standard names and production traits including growth and lifetime prolificacy in 370 Rambouillet, Columbia, and Polypay sheep.
*Results:* The *DRB1* *2001 haplotype was associated with increased weaning and mature weights, as well as average daily gain (Šidák $P < 0.05$; corrected for the number of haplotypes tested). Interestingly, the *2001 haplotype also showed a trend toward association with increased total number of lifetime lambs born (Šidák $P = 0.084$) and number of lambs born alive (Šidák $P = 0.084$). In contrast, the *DRB1* *0301 haplotype was associated with decreased mature weight (Šidák $P = 0.01$).
*Conclusions:* Since the *2001 haplotype was present in all three breeds, these results suggest there is at least one functional mutation in the region that influences growth and prolificacy traits that may be broadly present across several breeds. Furthermore, combined use of the similar *2001 and *0301 multi-marker haplotypes that nonetheless have opposing directions of production trait associations will enhance mutation discovery in this region. If undesirable alleles for underlying mutations can be identified, selective pressure against one or a small number of undesirable alleles may improve production with limited impact on MHC genetic diversity and infectious disease susceptibility.



## 1. Introduction

Sheep are a major domestic animal species for meat, milk, and wool products (Morris, 2009). The Major Histocompatibility Complex (MHC) of the sheep, designated as ovine Lymphocyte Antigen (OLA), harbors clusters of immunological genes involved in overall resistance/susceptibility of the animal to infectious diseases (Gao et al., 2010). Genetic loci in the MHC are organized to form distinct functional clusters as classes I, II, and III, which show considerable conservation among mammal species, most likely due to their importance for host-pathogen interactions (Glynn, 1988). Class I and II MHC genes encode cell-surface glycoproteins (MHC molecules) that present small peptide antigens to T-cells, thereby controlling both cell- and antibody-mediated immune responses. The MHC class III region consists of genes sandwiched between the MHC class I and II regions in placental mammals and may play a role in the innate immune system as opposed to the adaptive immune

*Abbreviations:* MHC, major histocompatibility complex; DRB1, DR beta 1; OLA, ovine lymphocyte antigen; DQB1, DQ beta 1; DNA, deoxyribonucleic acid; BLV, Bovine leukemia virus; SRLV, small ruminant lentiviruses; QTL, quantitative trait loci; cDNA, complementary DNA; RT-PCR, Reverse transcription polymerase chain reaction; SNP, single nucleotide polymorphism; GSTA1, glutathione S-transferase 1; GSTA4, glutathione S-transferase 4; °C, centigrade; BRWT,, birth weight; JWT, weight in the spring of the year born; WNWT, weaning weight; WT120, weaning weight adjusted to a constant 120 days; ASWT, weight collected late summer in the year born; SPWT3, weight of 3 years of age in spring; SPWT4, weight of 4 years of age in spring; FAWT3, weight of 3 years of age in fall; FAWT4, weight of 4 years of age in fall; ADG, average daily gain; LLB, total number of lambs born; LLBA, number of lambs born alive; LLW, number of lambs weaned; LLBW, cumulative ewe lifetime lamb birth weights; LLWW, lifetime lamb weaning weights; LFLWT, lifetime greasy fleece weight.

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system (Deakin et al., 2006). In addition, there are non-immunological genes in the MHC region, including components of the proteasome and iron metabolic pathways, among others (Janeway et al., 2001; Jiang et al., 2014).

Among sheep MHC class II genes, the *DRB1* and *DQB1* loci have been found to be highly polymorphic and this polymorphism is maintained by a form of balancing selection (Hughes and Yeager, 1998). In particular, exon 2 encodes the highly polymorphic antigen-binding site (Herrmann-Hoesing et al., 2008) where 106 *Ovar-DRB1* separate alleles have been identified (Ballingall et al., 1992; Fabb et al., 1993; Schwaiger et al., 1995; Kostia et al., 1998; Paterson, 1998; Nagaoka et al., 1999; Jugo and Vicario, 2000; Konnai et al., 2003a; Herrmann-Hoesing et al., 2008). Variation in the *Ovar-DRB1* gene may impact immune responses to pathogens, which may lead to variation in disease resistance. Numerous investigations have detected polymorphism of sheep MHC genes and described association with resistance or susceptibility to infectious diseases including studies on resistance to nematodiasis (Sayers et al., 2005; Stear et al., 2009; Valilou et al., 2015), bacterial diseases like footrot (Escayg et al., 1997) and viral diseases such as BLV-induced ovine lymphoma, small ruminant lentiviruses (SRLV: also called Maedi-Visna or ovine progressive pneumonia virus), and pulmonary adenocarcinoma viral diseases (Aida et al., 1995; Konnai et al., 2003b; Herrmann-Hoesing et al., 2008; Larruskain et al., 2010).

Besides the role of the MHC in immune resistance, quantitative trait loci (QTL) have been identified in or near the MHC region associated with economically important traits such as growth and meat quality in pigs (Wei et al., 2011), semen traits and growth rate of young bulls (Grignola et al., 1995), increased protein yield and fat percentage in cow milk (Batra et al., 1989; Sharif et al., 1999), and milk yield, body weight, and maximum daily gain in sheep (Gutierrez-Gil et al., 2009; Hadjipavlou and Bishop, 2009; Raadsma et al., 2009). These QTL may be the result of pleiotropic effects of some MHC genes on physiologic processes, or they might result from linkage disequilibrium with other nearby genes. However, only one microsatellite-based study with 249 ewes and 381 lambs, found an association of *Ovar-DRB1* polymorphism with any growth and reproduction traits in sheep (Geldermann et al., 2006), and that study only used one year of records instead of ewe cumulative lifetime production. However, ewe cumulative lifetime production is one of the most economically important metrics, especially since replacement costs are a substantial fraction of total costs for ruminant livestock production systems. To the best of our knowledge, no study has evaluated an association of lifetime production traits with *Ovar-DRB1* in sheep. Therefore the aim of this study was to investigate association between *Ovar-DRB1* haplotypes and individual and lifetime production traits in Rambouillet, Columbia, and Polypay sheep.

## 2. Methods

### 2.1. Animals and phenotypes

We sampled 370 Rambouillet, Columbia, and Polypay ewes from the Range Sheep Production Efficiency Research station in Idaho as described previously (Herrmann-Hoesing et al., 2008). Briefly, approximately equal numbers were chosen by breed and age (3, 4, 5, and 6 years in 2004) for a separate study on small ruminant lentivirus (Herrmann-Hoesing et al., 2008). In this analysis, the totals were: 129 Rambouillet, 117 Columbia, and 124 Polypay ewes. Phenotypes were recorded as individual traits and lifetime production traits (collected both before and after 2004), an accumulation of specific production traits. Individual ewe traits were defined as unadjusted birth weights (BRWT), unadjusted weights in the spring of the year born (JWT), unadjusted weaning weights (WNWT), weaning weight adjusted to a constant 120 days (WT120), unadjusted weights collected late summer in the year born (ASWT) and unadjusted weights twice a year during 3 and 4 years of age in spring and fall (SPWT3, FAWT3, SPWT4 and FAWT4). Average daily gain (ADG) was calculated as the difference between the unadjusted weight at weaning minus birth weight divided by an adjusted age of 120 days. For example, lambs younger than 120 days had an increased adjusted weaning weight and older lambs had a decreased adjusted weaning weight compared with unadjusted weaning weight. Fleece was shorn annually, and unwashed, greasy weight was recorded (LFLWT). Milk score (0 = none to 5 = very good) was a subjective assessment by trained staff on the amount of milk available shortly after birth in the final year of the ewe's productive life. Subjective udder (mammary gland) scores were collected each year with a 12 point scale in which 1 was removed from analysis because the ewe was not lactating, 2–3 were considered normal, and 4–12 were abnormal. Total number of lamb(s) born (LLB), number of lamb(s) born alive (LLBA), and number of lamb(s) weaned (LLW) were analyzed as cumulative lifetime counts. Cumulative ewe lifetime lamb birth weights were unadjusted (LLBW) and lifetime lamb weaning weights (LLWW) were adjusted to an average age of 120 days.

### 2.2. RNA isolation and genotyping of Ovar-DRB1

Because distinguishing expressed genes from pseudogenes is an issue especially in the MHC (Kumanovics et al., 2003), we used RNA to generate genotypes only from expressed genes. Details of RNA isolation and genotyping of *Ovar-DRB1* were given in a previous study (Herrmann-Hoesing et al., 2008) that focused on different traits. Briefly, total RNA was extracted from 2 ml whole blood using PAXtube technology following manufacturer's directions (Qiagen, Hilden, Germany) and was stored at −80 °C until needed. RNA quality was assessed by gel electrophoresis and 260/280 nm spectrophotometric analysis prior to RT-PCR, and RT-PCR positive and negative controls for each reaction set further confirmed input RNA quality of all samples. *Ovar-DRB1* allelic typing was conducted by cloning RT-PCR products spanning the peptide-binding site and sequencing, or by cDNA sequencing combined with use of electronic confirmation of known haplotypes (Stephens et al., 2001; Stephens and Donnelly, 2003; Herrmann-Hoesing et al., 2008), as previously described. The resulting haplotypes were matched to internationally accepted haplotype names (Ballingall et al., 2011), and these standardized names were used throughout the study.

### 2.3. Statistical analysis

Data were checked for normality before analyses using the UNIVARIATE procedure of SAS v9.4 (SAS Institute, Cary, NC). All individual and lifetime cumulative trait data were analyzed with a mixed model using the MIXED procedure of SAS v9.4. The model included fixed effects of breed, year of birth (corresponded to age in years for this study), and genotype, with a random effect of sire nested within breed. For discrete traits such as milk and udder scores, the GLIMMIX procedure of SAS v9.4 was used to examine association with *Ovar-DRB1* genotypes with the same fixed and random effects. A Šidák correction for multiple testing (Šidák, 1967) was applied on a haplotype basis for all haplotypes present in 30 or more individuals. The Šidák method for multiple testing correction makes the strong assumption of complete statistical independence, and it is therefore overly conservative in any set of analyses within which positive dependence occurs. For each statistical test, Šidák *P*-values were reported to account for multiple testing and nominal *P* values were reported for completeness and to allow for additional reader analyses, if desired.

## 3. Results

Associations of *Ovar-DRB1* haplotypes with the individual and lifetime production traits in an Idaho ewe flock consisting of Rambouillet, Columbia, and Polypay breeds were investigated in 370 sheep. From sequencing of cDNA products, 31 DRB1 haplotypes were identified in 370 ewes. Total numbers of animals possessing at least one copy of each

*DRB1* haplotype and overall breed-specific *DRB1* haplotype frequencies were presented in supplementary Table 1.

For individual ewe traits (excluding lamb production), the *DRB1* *2001 haplotype was significantly associated with JWT, WWT and ASWT after multiple testing (all Šidák $P < 0.05$; Table 1), and all three breeds carried this haplotype (supplementary Table 1). The *DRB1* *2001 haplotype also was nominally associated with WT120 ($P = 0.01$), ADG ($P = 0.01$), FAWT3 ($P = 0.04$) and showed a nominal trend toward association with FAWT4 ($P = 0.07$; Table 1). No additional significant associations with *DRB1* *2001 were found (supplementary Table 2). *DRB1* *0301 was nominally associated with WT120 ($P = 0.01$), WWT ($P = 0.01$), ADG ($P = 0.02$), ASWT ($P = 0.0081$), SPWT3 ($P = 0.0011$; Šidák $P = 0.01$), and FAWT3 ($P = 0.05$) (Table 2; supplementary Table 2). *DRB1* *0301 also showed a trend toward association with BWT ($P = 0.09$), JWT ($P = 0.08$), and SPWT4 ($P = 0.07$) (supplementary Table 2). However, this haplotype was detected only in Columbia and Rambouillet ewes (supplementary Table 1). Nominally significant association was observed between several additional haplotypes and ewe individual traits that were not significant after Šidák correction for multiple testing (supplementary Table 2). None of the haplotypes was found to be associated with milk and udder traits (all nominal $P > 0.05$) and therefore were not shown in supplementary Table 2.

For ewe lifetime lamb production traits, *DRB1* *2001 was associated with LLBA and LLW (Table 1). *DRB1* *0301 was significantly association with LLB ($P = 0.03$) and a trend toward association with LLW ($P = 0.09$) and LLWW ($P = 0.1$; Table 2). Additional nominally significant results that were not significant after Šidák correction for multiple testing are shown in supplementary Table 3.

Additionally, thirty-two ewes in the population (8.6%) were homozygous for *DRB1* where 2.4%, 1.6%, and 1.1% of the population were homozygous for *DRB1* *1102, *DRB1* *0701, and *DRB1* *0201, respectively (Supplementary Table 4). In terms of breed-specific haplotypes, *DRB1* *1401 was found only in Columbia ewes. *DRB1* *0902 and *DRB1* *0401 were only found in Rambouillet ewes. *DRB1* *2101 was found only in Polypay ewes (Supplementary Table 1). Summary statistics for individual and lifetime traits are in supplementary Table 5 are provided for context concerning the sheep population used.

## 4. Discussion

In our study we identified several associations between *Ovar-DRB1* haplotypes and weight gain at different life stages as well as ewe lifetime number of lambs born and born alive (Tables 1 and 2; supplementary Tables 2 and 3). Lambs that grow rapidly and reach market weights at younger ages, require a shorter feeding period, have improved feed efficiency, and therefore, increase producer profits. Further, lamb survival is a complex trait influenced by direct genetic, maternal genetic, and environmental effects, and genetic selection to improve this trait is possible (Everett-Hincks et al., 2014).

In at least two previous experiments, Rambouillet, Columbia, and Polypay breeds were clearly clustered separately based on genome-wide analysis of 50,000 SNP (White et al., 2012; Zhang et al., 2013). This study identified breed differences in production relevant phenotypes. Specifically, we identified differences in birth weight, fleece weight, and lifetime number of lambs born (Tables 1 and 2). These findings are consistent with breed standards for a semi-prolific breed (Polypay), a wool breed (Rambouillet), and a multipurpose breed (Columbia) and with previous work (Bromley et al., 2000; Bromley et al., 2001). The breed differences in genome-wide genotypes and multiple phenotypic measures highlight genetic associations with the *2001 haplotype that was observed in all three breeds (supplementary Table 1).

However, there is very little literature regarding *Ovar-DRB1* association with production traits in sheep. Geldermann et al. (2006) genotyped *OVA-DRB1* intron 2 microsatellite markers in 249 Merinoland ewes and found significant association with birth weight, weaning weight, and average daily gain. This is generally consistent with our findings, though growth trait associations reported here included more weights at older ages, in addition to the improved genetic markers from defined haplotypes. Major Histocompatibility Complex class II molecules are important cell-surface glycoproteins for the adaptive immune system because they present pathogen-derived peptides to CD4 + T lymphocytes (Duraes et al., 2013). While it is not immediately clear how MHC class II genes might be directly related with increase in body size, there are at least three possible explanations for indirect relationships of *DRB1* haplotype *2001 with growth and prolificacy traits shown here: 1) linkage disequilibrium with one or more other genes on ovine chromosome 20, 2) influence on susceptibility to one or more common pathogens with impact on production traits, and/or 3) less obvious mechanisms including downstream effects of inflammation.

The MHC is characterized by long, ancient haplotypes spanning many genes (Raymond et al., 2005). Thus, it is possible that the *DRB1* *2001 and *0301 haplotypes are in linkage disequilibrium with one or more functional mutations in or affecting other genes on ovine chromosome 20. The sheep QTL database (Hu et al., 2016) contains several significant QTLs associated with morphostructural phenotypes, average daily gain, body weight at different ages and maximum daily gain (Hadjipavlou and Bishop, 2009). In addition, consistent with our findings Geldermann et al. (2006) found significant association between *OVA-DRB1* intron 2 variants and number of lambs born and number of lambs weaned in a single production year from Merinoland breed ewes. However, most of these studies used microsatellite markers that cannot be directly transferred across populations because of the high microsatellite mutation rate even within families. In contrast, the multi-marker haplotypes composed of evolutionarily stable SNP in named *DRB1* haplotypes *2001 and *0301 are readily identifiable in different populations. Thus, the specific identification of these named haplotypes will facilitate validation of our findings across populations, which is important for adoption in breeding programs and for mutation discovery (White and Knowles, 2013). Phylogenetic analysis of 31 *Ovar-*

**Table 1**
Association of *Ovar-DRB1* *2001 haplotype with individual and lifetime ewe traits.

| Trait | Presence of haplotype | Absence of haplotype | Nominal *P*-value | Šidák *P*-value |
|---|---|---|---|---|
| JWT[a] | 30.78 | 28.84 | 0.0031 | 0.040 |
| WWT[a] | 40.61 | 37.99 | 0.0003 | 0.004 |
| ASWT[a] | 46.10 | 43.37 | 0.0003 | 0.004 |
| WT120[a] | 40.27 | 38.46 | 0.014 | 0.14 |
| ADG[a] | 0.29 | 0.27 | 0.013 | 0.14 |
| FAWT3[a] | 82.21 | 79.48 | 0.047 | 0.47 |
| FAWT4[a] | 83.20 | 77.14 | 0.079 | 0.68 |
| LLB[b] | 12.57 | 11.52 | 0.0097 | 0.14 |
| LLBA[b] | 10.51 | 11.47 | 0.01 | 0.14 |

JWT: June weight after birth, WWT: Weaning weight, ASWT: August–September weight in the year born, WT120: Adjusted weight to a constant 120 days, ADG: Average daily gain, FAWT3: Weight in September of 3 years old, FAWT4: Weight in September of 4 years old. LLB: Lifetime number of lambs born; and LLBA: Lifetime number of lambs born alive.
[a] Lamb count phenotype.
[b] Weight phenotype in kg.

**Table 2**
Association of *Ovar-DRB1* *0301 haplotype with individual and lifetime ewe traits.

| Trait | Presence of haplotype | Absence of haplotype | Nominal *P*-value | Šidák *P*-value |
|---|---|---|---|---|
| SPWT3[a] | 68.70 | 75.94 | 0.0011 | 0.01 |
| BWT[a] | 4.63 | 4.90 | 0.090 | 0.77 |
| JWT[a] | 27.53 | 29.08 | 0.087 | 0.73 |
| WT120[a] | 36.30 | 38.73 | 0.018 | 0.14 |
| WWT[a] | 35.85 | 38.34 | 0.014 | 0.14 |
| ASWT[a] | 40.95 | 43.75 | 0.0081 | 0.12 |
| ADG[a] | 0.26 | 0.28 | 0.020 | 0.27 |
| FAWT3[a] | 73.60 | 77.92 | 0.058 | 0.55 |
| SPWT4[a] | 68.70 | 75.94 | 0.071 | 0.68 |
| LLB[b] | 11.63 | 11.37 | 0.030 | 0.32 |
| LLW[b] | 10.73 | 10.60 | 0.094 | 0.77 |
| LLWW[a] | 8.75 | 8.63 | 0.10 | 0.81 |

SPWT3: Weight in April/May of 3 years old, BWT: Birth weight; JWT: June weight after birth, WT120: Adjusted weight to a constant 120 days, WWT: Weaning weight, ASWT: August–September weight in the year born, ADG: Average daily gain, FAWT3: Weight in September of 3 years old, FAWT4: Weight in September of 4 years old, LLB: Lifetime number of lambs born; LLW: Lifetime number of lambs weaned; and LLWW: Total weight of lambs weaned over a lifetime.
[a] Weight phenotype in kg.
[b] Lamb count phenotype.

*DRB1* haplotypes revealed three main branches called A, B and C with *2001 (previously known as *0404) and *0301 (previously known as *0141) haplotypes found in branch C (Herrmann-Hoesing et al., 2008). This makes the opposing directions of association (Tables 1 and 2) between these similar *DRB1* haplotypes more useful, because comparing similar extended haplotypes may help greatly reduce the search region for potential causal mutations. Additionally the region of ovine chromosome 20 near *Ovar-DRB1* harbors genes related with reproduction traits such as glutathione S-transferase 1 and 4 (*GSTA1* and *GSTA4*) which were associated with oocyte competence (Salhab et al., 2011) and granulosa cell development (Fayad et al., 2004), as well as genes related with growth traits like tenascin XB (*TNXB*) (Ajayi et al., 2014).

A second hypothesis to explain the observed associations could involve improved control of one or more common pathogens with consequent improved average growth. Indeed, the associations we observed with lifetime traits may suggest improved overall health for sheep bearing the *2001 haplotype. There are also several examples of data from the literature that may support this hypothesis. The *2001 haplotype was found only in healthy individuals but not in sheep with small ruminant lentiviruses (SRLV) or ovine pulmonary adenocarcinoma virus-related disease (Larruskain et al., 2010). Furthermore, the *2001 haplotype contains amino acid N74 that was previously associated with improved control of SRLV (reduced SRLV proviral concentration) (Herrmann-Hoesing et al., 2008). Both SRLV and pulmonary adenocarcinoma virus can affect growth (Arsenault et al., 2003; Bennett, 2003; Bennett and Ijpelaar, 2005). Association of *DRB1* variants with resistance to intestinal nematode infection were shown in several sheep breeds (Sayers et al., 2005; Stear et al., 2009; Shen et al., 2014; Valilou et al., 2015). Generally, increase in faecal egg count of nematodes is associated with decreased sheep performance in terms of body weight, wool production, and milk yield (Mavrot et al., 2015). For example, a population of Scottish Blackface sheep had QTLs in the vicinity of the *Ovar-DRB1* locus associated with parasitic resistance (Davies et al., 2006), daily gain, and body weight (Hadjipavlou and Bishop, 2009). These and other examples suggest that *DRB1* may be indirectly related to sheep production through improved pathogen control. If so, the six amino acid residues that differ between *2001 and *0301 may be implicated in differential presentation of important pathogen epitopes.

A third possibility might include downstream effects of inflammatory conditions, which can result in significant upregulation of MHC II molecules (Nagaraju, 2001). Skeletal muscles can be injured by their own contractions during everyday activities (Marino et al., 2011). The range ewes we evaluated are required to walk up and down mountains for forage and water, and the exertion may contribute to muscle inflammation and help explain the association of *Ovar-DRB1* variants with growth traits in our ewe population.

The MHC plays a critical role in the immune recognition of parasites and pathogens. There are possible associations of MHC loci with a number of quantitative traits linked to the fitness and behavior of individuals in natural populations (Piertney and Oliver, 2006). A genetic selection program based on MHC or nearby genes may cause undesirable effects on diversity maintained over long periods of balancing selection. However, Apanius' heterozygote advantage and frequency dependence theories (Apanius et al., 1997) suggest that eliminating the most susceptible alleles from the global population, but keeping other alleles, could be effective for livestock populations with minimal impact on overall MHC diversity. Indeed, Maillard et al. (2003) demonstrated a practical example of such selection and defeated a population-limiting infectious disease crisis in cattle. If underlying mutations affecting growth and reproductive performance can be found on ovine chromosome 20, elimination or reduction of some undesirable performance alleles could be implemented with limited impact on flock level MHC genetic diversity and infectious disease resistance.

## 5. Conclusion

In summary, our results define associations between *2001 and *0301 *Ovar-DRB1* haplotypes and growth and prolificacy traits across 3 different sheep breeds. Specifically, the *DRB1* *2001 haplotype was associated with increased weaning and mature weights, as well as average daily gain, while the *DRB1* *0301 haplotype was associated with decreased mature weight. Interestingly, the *2001 haplotype also showed a trend toward association with increased total number of lifetime lambs born and number of lambs born alive. These observed associations suggest the existence of one or more functional mutations on the *DRB1* *2001 and *0301 haplotypes which affect prolificacy and growth performance. Further studies will be required in order to understand sheep MHC genetics and identify functional mutations on ovine chromosome 20 involved in reproduction, prolificacy, growth, and other production traits.

## Conflict of interest

The authors declare they have no conflicts of interest.

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## Appendix A. Supplementary Tables 1–5

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.10.004.

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